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NCRR: Catalyst for Discovery

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From the Director

Advancing the Frontier of Stem Cell Research

The National Center for Research Resources (NCRR) has long supported pioneering studies—studies considered to be on the leading edge of the research frontier. These opportunities, often times considered high risk, can hold the promise of high societal payoffs. The research related to nonhuman embryonic stem (ES) cells is one such study.

More than 10 years of basic research in primate developmental biology by research teams led by Dr. John Hearn at the NCRR-funded Wisconsin National Primate Research Center enabled the first isolation and culturing of ES cells from nonhuman primates in 1995. Using knowledge gained from these studies, Dr. James Thomson and his colleagues took the conquest forward in 1998 when they isolated and propagated human ES cells. (See "Exploring the Promise of Stem Cells," page 5.)

While the potential for stem cell therapies appears to hold great promise, the research is truly on the leading edge of a new frontier. Before their potential can be assessed, substantial basic research must take place on the characterization, mechanisms of differentiation, and regulation of cellular process of ES cells.

To advance the frontier, NCRR, along with five other components of the National Institutes of Health (NIH), has issued infrastructure enhancement awards to increase the capacity for basic research using human ES cells for preclinical investigations. The awards, which support entities listed on the NIH Human Embryonic Stem Cell Registry, are designed to increase the supplies and access to cells that are self-renewing and well-characterized for quality controls.

The overall objective of the support is to ensure that investigators have access to a sufficient quantity of cells for research and testing in multiple laboratories. This objective will be accomplished by providing funding for expansion, testing, quality assurance, and distribution of cell lines listed on the human ES cell registry. The development of quality controls to monitor the laboratory performance of the human ES cells also is an integral part of the initiative. This research, like much of the biomedical inquiry process in its early stages, requires investigators to cross check and validate their results.

The ES cell research opportunities once again highlight the importance of well-developed infrastructure to advance a new research frontier. Like the early work on ES cells at the Wisconsin National Primate Research Center, advances in biomedical science depend on state-of-the-art research environments. At their best, such environments not only include novel research tools and technologies but also facilitate collaboration among scientists and sharing of expertise. Through these infrastructure enhancement awards and other sources of research support, NCRR facilitates discovery by providing the resources that help researchers explore the promise found on today's biomedical frontier.

Judith L. Vaitukaitis, M.D.
Director, NCRR

NCRR Reporter

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Cover: Now that the federal government has sanctioned research on approved lines of human embryonic stem cells—like the rounded, dense cell clusters shown growing on a blanket of flat, elongated "feeder" cells—NCRR is helping to fund necessary infrastructure for testing and distributing these cells. (Photo courtesy of Dr. James Thomson, University of Wisconsin-Madison)

NCRR Reports

Research findings with NCRR support

Antibody Treatment Arrests Diabetes

Researchers have shown that an antibody designed to prevent the activation of certain immune cells may be an effective treatment for patients with newly identified type 1 diabetes, a condition in which the body's own immune system destroys insulin-secreting cells in the pancreas. At NCRR-funded General Clinical Research Centers and other sites around the country, scientists administered a two-week course of antibody treatment to patients who had been diagnosed with type 1 diabetes within the previous 6 weeks. Results showed that the antibody treatment maintained or improved insulin production in 9 of 12 patients for at least a year, while insulin production declined in 10 of 12 untreated patients. Treatment side effects were minor.

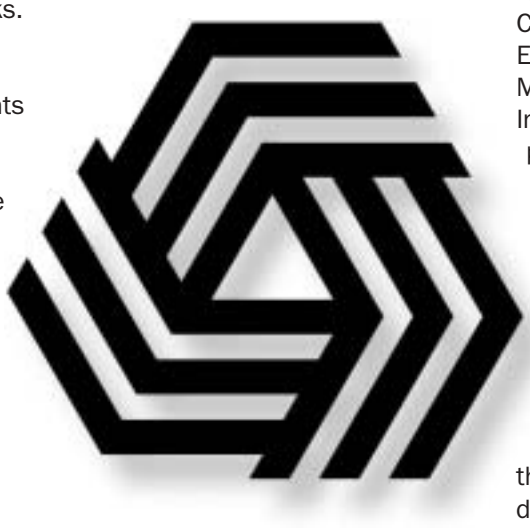
The researchers speculate that the antibody treatment may enhance protective immune responses while stifling immune responses that ultimately cause annihilation of insulin-producing pancreatic cells.

—*New England Journal of Medicine* 346:1692-1698, 2002.

Scientists Identify "Gravity Genes"

In the early 1960s, Dr. Jerry Hirsch at the University of Illinois at Urbana-Champaign launched the field of behavioral genetics by demonstrating that genetic differences in the fruit fly *Drosophila melanogaster* influence the insect's response to gravity. Now, four decades later, scientists have identified three genes that play a role in this behavior.

To show that *Drosophila* have gravity-responsive genes, Dr. Hirsch ran his flies through a series of tubular mazes that required insects to make sequential choices as to whether to move up or down. Most flies chose randomly, but some exhibited clear preferences for the upper or lower tubes, a behavior Dr. Hirsch dubbed "geotaxis." By consecutively breeding selected flies, Dr. Hirsch created lines of insects that consistently preferred upward or downward movement, which suggested that genes must be influencing their behavior. However, the responsible genes were not identified until this year, when scientists employed a new research tool known as cDNA microarray analysis, or DNA chips. Using this approach, which identifies the multiple genes expressed in specific organs such as the brain, the scientists were able to narrow their search for geotaxis genes to four candidates. Then using flies with mutations in these four genes, plus flies with mutations in five control genes, the scientists verified that three of the four candidate genes did indeed influence geotaxis behavior. Some of the fly mutants were obtained from the NCRR-funded Bloomington *Drosophila* Stock Center in Indiana. This approach for pinpointing genes may also prove useful for discovering additional behavior-associated genes.



—*Nature Genetics* 31:349-353, 2002.

Cancer Spreads in Tumor Margins

Scientists have long suspected that lymphatic vessels both in and around tumors might serve as a conduit for metastasis, or the spread of cancer cells. Now a research team has shown that this long-held hunch may be only partly correct. Investigators at the Massachusetts General Hospital in Boston, including a researcher now at the NCRR-funded Center of Biomedical Research Excellence in Angiogenesis at the Maine Medical Center Research Institute, have found that the lymphatic vessels surrounding—but not within—tumors are culpable in the dispersal of cancer.

To prove this point, the researchers created tumor cell lines that overexpressed vascular endothelial growth factor-C (VEGF-C), a chemical known to stimulate the growth of lymphatic vessels, and implanted the cells in the hind limb of immunodeficient mice. Compared to mice that received tumor-cell implants that generated normal amounts of VEGF-C, the mice with enhanced VEGF-C production had more extensive spreading of tumors via the lymph system. The scientists also observed a proliferation of functioning lymphatic vessels surrounding the tumors, in the so-called tumor margins, whereas vessels within the tumors were collapsed or blocked. The scientists concluded that metastasis occurs via lymphatic vessels in tumor margins and suggested that drugs targeting VEGF-C might inhibit the spread of cancer.

—*Science* 296:1883-1886, 2002.

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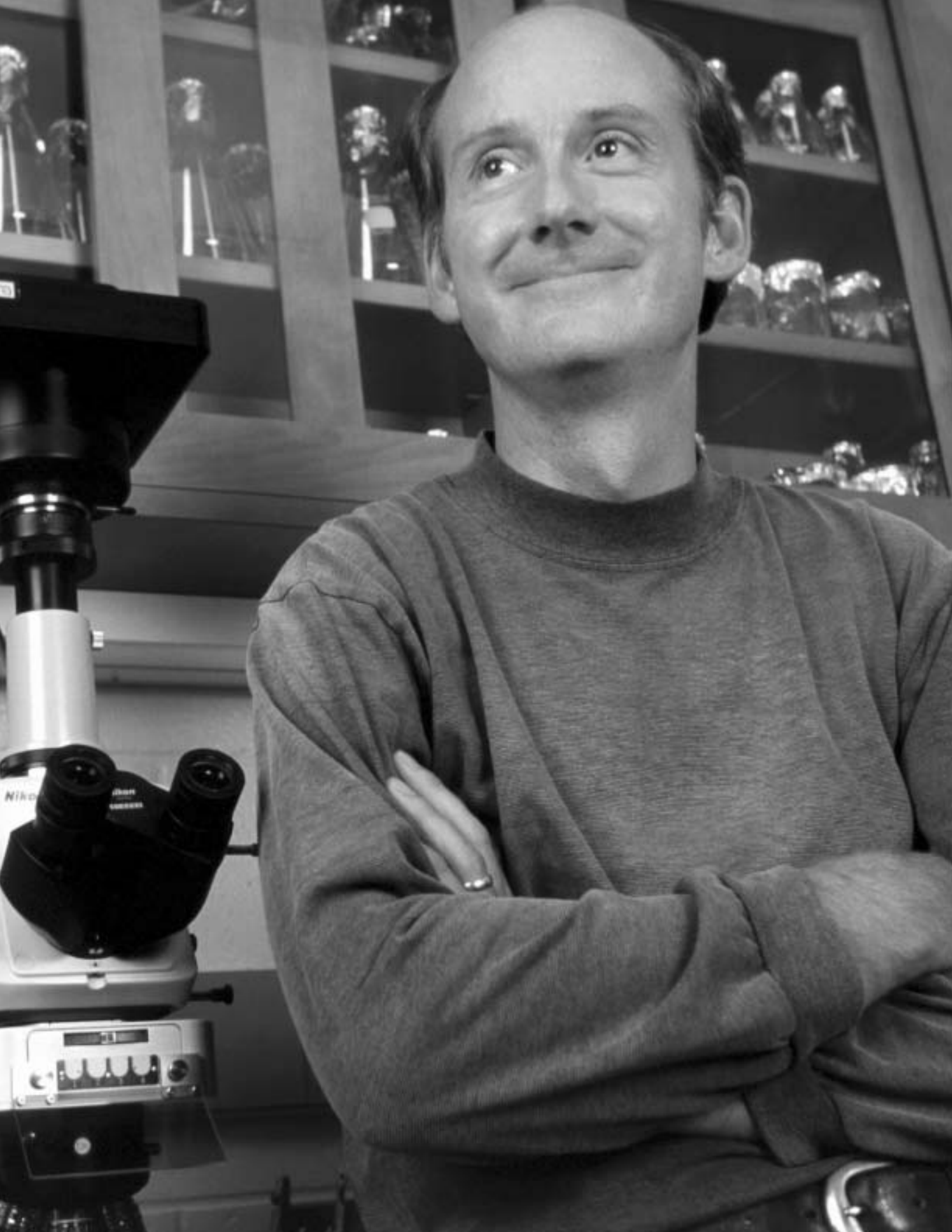

Exploring the Promise of Stem Cells

by Brenda Patoine

Stem cells have captured the world's attention for their potential use as therapies for devastating conditions such as Parkinson's disease, diabetes, and even heart disease. With their capacity to generate every cell type in the body, stem cells have been hailed as virtually unlimited sources for tissue and organ replacements, potentially eliminating the need for organ donors. Yet, for all the hype and hope surrounding these versatile cells, any clinical application is, by all accounts, years or even decades away. Scientists are still struggling to understand how embryonic stem (ES) cells generate daughter cells that can differentiate into multiple cell types. And researchers have not yet perfected the conditions for producing large quantities of these cells in an undifferentiated state. All of these hurdles must be overcome before the true promise of ES cells can be realized. "To make good judgments about how embryonic stem cells function and differentiate, we first need to know how to expand their production and select the best quality control criteria," says Dr. Anthony Hayward, director of NCRR's Division of Clinical Research.

Dr. James Thomson and his colleagues were the first to isolate embryonic stem cell lines from nonhuman primates and from humans. *(Photo by Jeff Miller, University of Wisconsin-Madison)*

Toward that end NCRR, along with five other NIH institutes, announced in April 2002 the first grants in its Infrastructure Enhancement Awards in human ES cell research. The two-year grants are designed to enable the laboratories that originally derived human ES cells to expand, test, and distribute their cells to qualified investigators. As of September 2002, five laboratories that have cell lines on the NIH Registry had received awards totaling more than $2.1 million: the University of California, San Francisco (UCSF); the Wisconsin Alumni Research Foundation in Madison; the Karolinska Institute in Sweden; BresaGen, Ltd. in Athens, Georgia; and ES Cell International Pte. Ltd., of Singapore and Melbourne, Australia.

NCRR has long supported ES cell research in animals, most notably the pioneering studies conducted by Dr. James Thomson, Dr. John Hearn, and their colleagues at the Wisconsin National Primate Research Center (NPRC). In 1995 the researchers reported the first isolation and culturing of ES cells from a nonhuman primate—the rhesus macaque. The researchers empirically determined that ES cells could be plucked from a sphere of embryonic cells, known as the blastocyst, and grown on a blanket of mouse connective tissue cells called fibroblasts. These "feeder cells," which secrete factors that support the growth of the ES cells and prevent them from differentiating, proved to be the critical element in producing rhesus ES cell cultures.

Importantly, cultured rhesus ES cells appeared to be capable of differentiating into the various cell types of the body. When treated with certain chemicals in culture or injected into immunodeficient mice to avoid problems with immune rejection, the ES cells differentiated into cells found in bone, muscle, nervous system, and other tissues. (For more information, see the *NCRR Reporter*, September/October 1995, pp. 4-7.)

Using knowledge gained from their nonhuman primate studies, Dr. Thomson and his colleagues took a significant step forward in 1998, when they isolated and propagated ES cells from humans. "The monkey studies were certainly a useful pre-

lude to the human work," comments Dr. John D. Harding, a health scientist administrator in NCRR's Division of Comparative Medicine, which supports the network of National Primate Research Centers across the country. "The Wisconsin studies demonstrated early on the feasibility of deriving embryonic stem cells from primates."

As in their nonhuman primate work, Dr. Thomson and his colleagues derived their human ES cell lines from cells formed only days after the egg was fertilized and started dividing. Shortly after the Wisconsin researchers announced their accomplishment, researchers at Johns Hopkins University reported that they had also cultured human ES cell lines, although theirs were derived from primordial germ cells—precursors of human sperm and eggs—taken from medically aborted 5- to 8-week-old embryos.

Scientists say federal support of stem cell infrastructure allows NIH, rather than a commercial entity, to "set the bar" for quality assurance of distributed cells.

Although these initial studies of human ES cells were privately funded, in August 2001 the U.S. government began to allow the use of federal funds to support research with approved, pre-existing human ES cell lines, including several derived by Dr. Thomson's laboratory. These cell lines are listed in the NIH Human Embryonic Stem Cell Registry (http://escr.nih.gov).

Scientists say federal support of stem cell infrastructure is critical because it allows NIH, rather than a commercial entity, to "set the bar" for quality assurance of distributed cells, says Dr. Keith Yamamoto, vice dean for research at UCSF School of Medicine. "When we know that everyone is starting with the same material, we will be better able to compare experiments from different laboratories," he says.

Although some of the institutions listed in the stem cell registry had begun shipping human ES cells prior to the infrastructure grants, federal funding is expected to dramatically enhance distribution. The infrastructure grants are designed to meet the individual needs of each institution, whether those needs are to rent space, purchase incubators, or add staff, says Dr. Hayward. "We're simply trying to give these laboratories enough money to enable them to switch from exclusively producing cells for their own research to also producing cells for other laboratories," he says.

The infrastructure grants also require that laboratories ship at least 200,000 cells to researchers who request them. This will provide scientists with enough cells to have a "master stock" of their own to propagate while using some of the cells for experiments, says Dr. Hayward. Before shipping the cells, scientists analyze them for certain cell-surface markers that identify the cells as both undifferentiated and capable of differentiating, while other markers detect chromosomal abnormalities. These various quality assurances are critical because the cells are expected one day to be used in the treatment of degenerative diseases.

A major barrier to using ES cells clinically has been the requisite use of mouse feeder cells in their production. Typically, human ES cells are grown on a layer of mouse fibroblasts, which provide still-unidentified growth factors that maintain the cells in an undifferentiated state. Unfortunately, reliance on mouse cells opens the door to potential transmission of pathogens, particularly animal viruses, from the feeder cells to the human ES cells. But now, in the September 2002 issue of *Nature Biotechnology*, scientists from ES Cell International describe a new technique that uses human,

Embryonic stem cells can be isolated after a fertilized egg (1) develops into a ball of cells called a blastocyst (2). From inside a blastocyst, the inner cell mass is extracted (3) and deposited (4) on a thin blanket of feeder cells, which allow the undifferentiated stem cells to proliferate. *(Courtesy of the University of Wisconsin-Madison)*

rather than mouse, fibroblasts as feeder cells, an advance that is important for developing cells that can be used therapeutically. The researchers first cultured human fibroblasts from adult fallopian tubes or from fetal muscle and skin. A layer of these feeder cells, or the solution in which they were grown, was then used to nurture cultures of human ES cells. With either the feeder cells or the solution, the human ES cells grew at least as well as they did with mouse feeder cells, and they retained all the expected characteristics of stem cells.

Aggressive research programs are already underway to test the usefulness of stem cell transplant strategies in animal models of Parkinson's disease. Other neurological disorders that might prove treatable with stem cells include stroke, spinal cord injury, amyotrophic lateral sclerosis (Lou Gehrig's disease), and Alzheimer's disease. In one recent study, Dr. Thomson and his colleagues succeeded in transforming human ES cells into transplantable neuronal precursor cells in vitro, an early step toward potential repair of brain tissue.

While disease treatments based on human ES cells are still many years off, Andrew Cohn, spokesman of the Wisconsin Alumni Research Foundation (WARF), believes that one of the first commercial applications of these cells will be to test drug candidates. "As soon as we can get purified heart tissue, I am confident that drug companies are going to want to use it to screen compounds," he says. Besides heart tissue, the University of Wisconsin-Madison has programs to develop blood, pancreatic, and neural cells. If potential drugs can be tested in vitro on normally functioning organ tissue, scientists might be better able to evaluate their effects.

Once the therapeutic potential of ES cells is determined, any treatments will likely be evaluated in the NCRR-supported General Clinical Research Centers (GCRCs), says Dr. Hayward. "The national network of GCRCs offers the research infrastructure needed to test new therapies," he says. "The GCRCs provide investigators with access to sophisticated laboratories and the specialized support staff needed to do the highest quality research."

Neurological disorders that might prove treatable with stem cells include stroke, spinal cord injury, Lou Gehrig's disease, and Alzheimer's disease.

For now, the field of human ES cell research is wide open. "If people are interested in going into this area of research, there are no longer many roadblocks," says Mr. Cohn. "NIH has made research money available, and cells can now be obtained from WARF/WiCell Research Institute and other sites. The more people working in this field, the faster discoveries will be made, and that will be good for us all."

The stem cell research infrastructure enhancement awards are funded by the Division of Clinical Research of the National Center for Research Resources; the National Heart, Lung, and Blood Institute; the National Institute on Aging; the National Institute of Child Health and Human Development; the National Institute of Diabetes and Digestive and Kidney Diseases; and the National Institute of Mental Health.

For more information about the NCRR Division of Clinical Research, visit www.ncrr.nih.gov/clinical_rsrch.asp.

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Research Highlights

Crossing the Channel

The world is filled with selective passageways that enable or prevent movement. From tollbooths to airport security to doors unlocked by an employee badge, selective passageways allow entry only when certain criteria are met. On a vastly smaller scale, selective passageways also play a critical role in human health. The outer membrane of every living cell—in organisms ranging from bacteria to humans—is riddled with tiny pores known as ion channels, which are specially designed to permit selective and rapid passage of specific electrically charged atoms, or ions. These specialized cellular channels are so critical to life that their malfunction can trigger a host of human disorders, including epilepsy, myesthenia gravis, cystic fibrosis, hypertension, and cardiac arrhythmias.

Discovering how ion channels operate has been the mission of Dr. Roderick MacKinnon, professor of neurobiology and biophysics at The Rockefeller University in New York City and Investigator in the Howard Hughes Medical Institute. Although the existence of ion channels has been generally acknowledged for nearly half a century, it wasn't until 1998 that Dr. MacKinnon and his colleagues gave the world its first detailed look at the molecular architecture of a selective ion channel—a simple bacterial channel selective for positively charged potassium ions. And it wasn't until this year, in May 2002, that scientists were able to see the structural details of how a specialized "gated" ion channel must operate. In another breakthrough finding, Dr. MacKinnon's research team deciphered the three-dimensional (3-D) structure of bacterial chloride channels, offering the first-ever atomic-scale glimpse of a negative-ion channel (see the *NCRR Reporter*, Summer 2002, p. 3).

Dr. Roderick MacKinnon depended on state-of-the-art tools in X-ray crystallography and mass spectrometry to uncover the structural details of selective ion channels. *(Photo courtesy of The Rockefeller University)*

These and other dramatic advances in understanding ion channel architecture were reported in relatively rapid succession, within a 5-year timeframe, by the Rockefeller scientists. Their success has depended not only on the dedication and creativity of Dr. MacKinnon's research team but also on the ready access to shared instrumentation and expert staff at the NCRR-supported biomedical technology resource centers—particularly the National Resource for Mass Spectrometric Analysis of Biological Macromolecules, headed by Dr. Brian Chait at Rockefeller University, and the Macromolecular Diffraction Biotechnology Resource (MacCHESS), located at the Cornell High Energy Synchrotron Source in Ithaca, New York. Dr. MacKinnon's team collaborated with colleagues in Dr. Chait's mass spectrometry resource to isolate and analyze ion channel components so that they could be more readily crystallized. And at MacCHESS, exceptionally brilliant X-rays were trained on the crystallized ion channels to expose their 3-D structures in unprecedented detail. "Our collaborative studies of the crystal structures could not have occurred without the equipment and instruments purchased with NCRR support, both in Brian Chait's resource and at MacCHESS," says Dr. MacKinnon.

By determining the 3-D structures of a variety of ion channels, the Rockefeller scientists have answered questions that had puzzled biologists since the 1950s, when the Nobel Prize-winning studies of A. L. Hodgkin and A. F. Huxley first suggested that selective ion channels must exist, although they were too small to be seen. How else to explain their observations that sodium and potassium ions rapidly traverse the cell membrane—in a predictable and controlled fashion—when an electrical signal travels down a neuron? Further research led scientists to conclude that ion channels must come in a variety of shapes and sizes that allow them to discriminate among various ions, although the structural details remained elusive.

Less than a decade ago, Dr. MacKinnon decided to attempt a feat that many considered virtually impossible—that is, solving the ion channel structure via X-ray crystallography, perhaps the most powerful technique available for analyzing the 3-D molecular structures. (For more information, see the *NCRR Reporter*, Spring 2002, pp. 21-23.) But to employ this approach, sample molecules must first

be crystallized, which presented a significant roadblock. Each ion channel is a multiprotein complex made up of unwieldy molecules that are difficult to stack and pack into the orderly crystal lattice needed for X-ray analysis.

Undaunted, Dr. MacKinnon and his colleagues chose a simple potassium channel from the bacterium *Streptomyces lividans* for their initial crystallization efforts. In collaboration with Dr. Chait, the scientists generated and purified relatively large quantities of the channel protein, which has sequence similarity to all known potassium channels. Some of the purified proteins were placed into artificial membranes for studies of channel function, which helped to pinpoint critical channel regions. The scientists then used enzymes to trim away nonessential or ungainly protein regions. Mass spectrometry greatly facilitated these efforts, Dr. MacKinnon says, since the advanced instrumentation and expertise in Dr. Chait's resource enabled rapid analysis of protein fragments and could readily determine if enzyme treatment had harmed the protein structure. (For more information about mass spectrometry, see the *NCRR Reporter*, Spring 2002, pp. 16-19.)

Dr. MacKinnon attempted a feat that many considered virtually impossible—solving the ion channel structure via X-ray crystallography.

With suitably trimmed proteins in hand, the scientists made dozens of crystallization attempts, and mass spectrometry was repeatedly used to assess protein integrity in the many crystals. "Before Dr. MacKinnon travels to the synchrotron to analyze the samples, he needs to know that he has high-quality crystals. Time on the synchrotron is very precious," says Dr. Chait. Indeed, synchrotron radiation is considered such a valuable tool by the biomedical community, and is in such high demand, that most synchrotron resources have tight schedules and long waiting lists of scientists who wish to gain access. Poor crystals make poor use of this valuable resource, Dr. Chait notes.

At the MacCHESS resource, the brilliant synchrotron X-rays were focused on the crystallized proteins, and computers analyzed how the X-rays were deflected, or diffracted, as they passed through. Based on these diffraction patterns, computational analyses deciphered the protein's three-dimensional structure at a resolution of 3.2 angstroms, which revealed atomic-scale details never before seen.

Since these structural findings were published in 1998, the Rockefeller scientists have used similar techniques and resources to solve a variety of ion channel structures and elaborate on the details of the original one. One recent puzzle solved by the MacKinnon group involves the gating mechanism that opens and closes many ion channels. A variety of gated channels exist, including ligand-gated channels, which swing open when signaling molecules unlatch the gate, and voltage-gated channels, which open in response to electrical stimuli. The Rockefeller scientists managed to crystallize an intact and open ligand-gated potassium channel, which is opened via calcium binding. Synchrotron analyses revealed a ring-shaped structure, made up of four identical double-protein subunits, at the channel's base. In the cell, this structure lies just inside the membrane. Calcium binding provides the chemical energy needed to stretch open the gate like the mouth of a balloon, thereby enabling potassium entry.

Drs. MacKinnon, Chait, and their colleagues are now pursuing the structural details of voltage-gated mechanisms, which may prove useful in designing treatments for so-called channelopathies such as the rare, sometimes fatal heart disorder known as long QT syndrome. In this disorder, potassium channels in heart muscle cells behave abnormally, due to either mutations in channel proteins or the binding of certain drugs to the channel. "Like many channel-related disorders, long QT syndrome is caused by a mix of factors that we're still trying to understand," comments Dr. MacKinnon. "One approach that we're now pursuing is to study how drugs that can cause the syndrome bind to the potassium channel."

—Victoria L. Contie

This research is supported by the Division of Biomedical Technology of the National Center for Research Resources, the National Institute of General Medical Sciences, and the Howard Hughes Medical Institute.

For more information about the NCRR Division of Biomedical Technology, see www.ncrr.nih.gov/biomedical_tech.asp.

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The Hormone That Harms Diets

Before his operation, Jim Rudolph weighed 460 pounds and spent most of his waking hours preoccupied with food. "I used to drive my wife nuts. I'd be thinking about upcoming meals before I finished the one I was eating," he says. "At breakfast, I'd be asking her 'What's for lunch?' 'What's for dinner?'" As a result of his prodigious weight, Rudolph was barely able to take care of himself. He needed help putting on his shoes and taking a bath, and he had to use an electric cart to go shopping.

Then three and a half years ago, Rudolph underwent an operation called a gastric bypass, in which the esophagus and a small adjoining area of stomach are attached near the beginning of the small intestine. After the surgery, Rudolph found that he was no longer preoccupied with food. "Now I'm not driven by hunger," he says. "It's more like I just run out of steam and need to eat something to keep going." His weight dropped to 260 pounds, and he is leading a more active life.

Rudolph is one of an increasing number of people who are getting gastric bypass operations to treat extreme obesity. Although studies showed that the operation was more effective than other procedures for weight loss, physicians did not fully understand how the operation worked. Now a study conducted at the NCRR-funded General Clinical Research Center (GCRC) at the University of Washington in Seattle suggests that gastric bypass triggers weight loss in part by reducing the blood levels of a hormone called ghrelin.

Discovered just three years ago by scientists in Japan, ghrelin initially was of interest because of its ability to stimulate the release of growth hormone. Then researchers found that injecting ghrelin into rodents increased their food intake and promoted fat accumulation, and infusing the hormone into their brains activated nerve cells known to help regulate feeding behavior. Later, investigators in the United Kingdom determined that injecting the hormone into people caused them to eat 30 percent more food at an all-you-can-eat buffet.

These findings suggested that rising ghrelin levels might be the hunger signal that triggers eating, or at least a major component of that signal. Endocrinologist Dr. David Cummings at the University of Washington reasoned that if ghrelin was indeed the mediator of mealtime hunger, then circulating ghrelin levels should rise right before a meal and fall shortly after eating. At the University of Washington GCRC, Dr. Cummings and his colleagues investigated this theory by collecting 38 measurements of ghrelin levels in healthy volunteers over a 24-hour period. They found that ghrelin levels rose by an average of 78 percent one to two hours before the onset of each meal and fell within one hour after eating. Also, peak ghrelin levels were higher than the level produced by administering the smallest ghrelin dose that stimulates eating. "The data are fairly compelling that ghrelin plays a role in triggering ordinary premeal hunger and therefore participates in the decision to start a meal," says Dr. Cummings.

Next, the investigators measured ghrelin levels over 24-hour periods in two groups of obese people who had lost weight, one group from dieting and the other from gastric bypass surgery. In the group that had lost weight by eating a low-calorie diet, ghrelin levels rose before each meal and fell afterward, just as they had in the normal-weight volunteers. However, baseline ghrelin levels for the 24-hour period were higher in the dieting group than in the normal-weight volunteers. For most of the dieters, the more weight that was lost, the more baseline ghrelin levels increased.

"Generally, dieters will tell you that they get hungrier after they lose weight, which may be why people tend to regain their weight," says Dr. Cummings. "Increased ghrelin levels may be one of the mechanisms behind this increased hunger."

In contrast, the overall ghrelin levels in the gastric bypass patients were so low that they could hardly be

After his gastric bypass operation, Jim Rudolph dropped 200 pounds and several pants sizes. New evidence suggests that this type of operation alters hormone levels that control hunger. *(Photo courtesy of James Rudolph)*

measured. Also, the levels were perfectly flat throughout the day, with no peaks and valleys around mealtimes.

A gastric bypass operation creates a shunt around most of the stomach and all of the first part of the small intestine, which are the areas where most of the ghrelin is produced. As a result, food never contacts the ghrelin-producing cells in this region. "Something about this rewiring of the gastrointestinal tract seems to silence the principal ghrelin-producing cells," says Dr. Cummings. Coincident with this drop in ghrelin is a generalized loss of hunger. Studies have shown that gastric bypass patients eat fewer meals and fewer calorie-dense foods, such as cake and ice cream, not through an exertion of willpower but simply because they are less hungry. "The suppression of ghrelin after gastric bypass may be a reason why hunger does not rise after this procedure but in fact falls," says Dr. Cummings.

The GCRC played a critical role in these studies, according to Dr. Cummings. The center allowed the investigators to conduct frequent blood samplings, which would have been difficult to do in any other setting. Also, the research dieticians at the center monitored food intake and prepared special diets. To measure percentages of body fat, the scientists used the center's underwater weighing equipment.

Rising ghrelin levels might be part of the hunger signal that triggers eating.

Pharmaceutical companies are currently developing drugs that block ghrelin action in the brain, hoping that these drugs may facilitate weight loss. Whether they do or not may depend on the type of obesity, according to Dr. Cummings. He predicts that ghrelin blockers should work "like gangbusters" in patients who are obese because of Prader-Willi syndrome, a rare genetic disorder. His group found that ghrelin levels in these patients were the highest yet measured in humans, which implicates ghrelin as the driving force behind their intense eating. In contrast, ghrelin levels in ordinary obesity are actually lower than normal. These reduced levels may be part of the body's reaction to obesity, rather than a cause, he suggests.

Whether a ghrelin blocker would help people with ordinary obesity lose weight is unclear, says Dr. Cummings. "It might, especially since ghrelin tends to rise when you lose weight through dieting. If you could block that rise, you might cripple one of the body's ways of fighting against weight loss. My own prediction is that a ghrelin blocker will not make you lose a lot of weight by itself but might make it easier to lose weight through dieting and exercise."

—Steven Stocker

Dr. David Cummings and his colleagues discovered that the hormone ghrelin might play a role in triggering premeal hunger. *(Photo by Eden Palmer, Veterans Affairs Puget Sound Health Care System)*

This research is supported by the Division of Clinical Research of the National Center for Research Resources, the National Institute of Diabetes and Digestive and Kidney Diseases, the Department of Veterans Affairs, and the Burroughs Wellcome Fund.

For more information about the NCRR Division of Clinical Research, see www.ncrr.nih.gov/clinical_rsrch.asp.

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Critical Resources

Surveying the Sea Urchin Genome

For more than 30 years, Dr. Eric Davidson and his research team at the California Institute of Technology in Pasadena have steadily teased apart the molecular genetics of a common ocean-dwelling creature, the purple sea urchin *(Strongylocentrotus purpuratus)*, searching for the regulatory mechanisms that underlie its development from fertilized egg to free-swimming larva. The Caltech scientists have long recognized what a growing segment of the biomedical community is beginning to appreciate—that the sea urchin is a unique and tractable organism with enormous potential for shedding light on the most basic biological processes, including many that are relevant to human health. Indeed, the National Human Genome Research Institute recently selected the sea urchin as a top priority for genome sequencing, along with the genomes of the chimpanzee, dog, cow, and other animals of scientific interest.

According to Dr. Davidson, sea urchin research is now poised to make important contributions to broad biomedical areas, including cell biology, molecular embryology, fertilization biology, evolution, and the regulation of gene expression. To help ensure that the biomedical community has ready access to genetic, biologic, and database-related tools that will enable such studies, NCRR began to fund the Sea Urchin Genome Resource in 2000. Headed by Dr. Davidson, who is the Norman Chandler Professor of Cell Biology at Caltech, the resource prepares and distributes high-density arrayed filters, or macroarrays, that contain large complementary DNA (cDNA) libraries from the sea urchin. The macroarrays allow scientists to rapidly assess and compare gene expression in various stages of sea urchin development and in various cell types.

Each animal produces millions of eggs or sperm, providing an almost unlimited supply of basic genetic material.

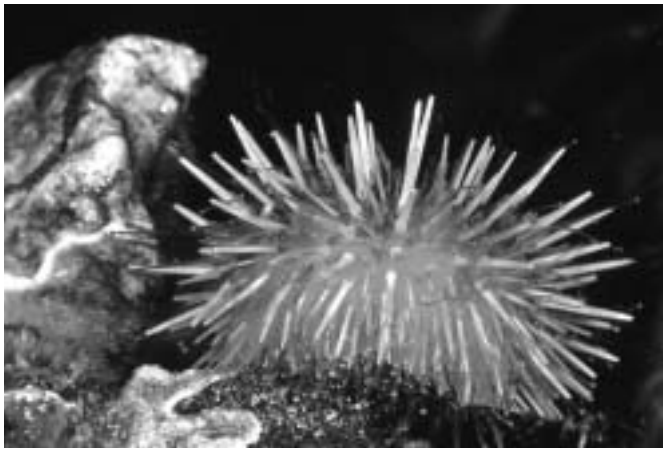
With more than a decade of NCRR funding, Dr. Davidson has developed advanced culture and molecular techniques for studying *S. purpuratus* and has produced an inbred line of animals, which is now up to an eighth generation. Inbreeding helps to create a more uniform gene pool, allowing scientists to more readily pinpoint the location of each nucleotide in a gene.

As a laboratory animal, the sea urchin is appreciated for its versatility and expediency. Each animal produces millions of eggs or sperm, providing an almost unlimited supply of basic genetic material; even rare proteins can be fairly easily isolated from this abundance of raw material. Sea urchin embryogenesis takes several days, beginning with a fertilized egg and ending with the creation of a transparent larva. Its transparency simplifies experimental manipulation and observation.

Like many other marine invertebrates, *S. purpuratus* undergoes "maximal indirect development," meaning that the process by which an embryo transforms into a larva is utterly unlike the process by which a larva becomes a juvenile. Each process also utilizes an entirely different set of precursor cells. Molecular developmental biologists have taken advantage of these distinct processes, focusing much of their research on the first step, embryogenesis, which is simpler and more easily manipulated.

Much of the day-to-day work in Dr. Davidson's laboratory involves microinjecting particular DNA segments into sea urchin eggs, modifying small bits of sequence at a time, and then observing the consequences. The scientists also are able to block expression of any given gene and then measure the resulting effects to other related genes. In this way, the scientists have delineated the complex interactions by which genes regulate other genes during the development of specialized tissues in the first 24 hours of life. Perhaps 50 of the 8,500 genes expressed in the sea urchin embryo are involved in this

Dr. Eric Davidson heads the new Sea Urchin Genome Resource, which distributes cDNA-based macroarrays to qualified researchers worldwide. *(Courtesy of the Laboratory of Embryonic Gene Expression, California Institute of Technology)*



The purple sea urchin* (Strongylocentrotus purpuratus) *offers many advantages for developmental biologists studying gene regulation. *(Photo by Laura Francis, National Oceanic and Atmospheric Administration)*

Sea urchins are close enough to humans to make comparison worthwhile, yet distant enough to provide insight into the course of evolution over the last 540 million years.

process, and many of these 50 genes code for proteins known as transcription factors, which simultaneously activate or inhibit large groups of genes.

As elsewhere in the life sciences, making sense of so much data and so many interconnections depends heavily on modeling and computers. To aid such analyses, the Caltech scientists collaborated with other research teams to develop three software programs—Netbuilder, FamilyRelations, and an early version of BioArray—that are available online and can be accessed by biomedical researchers free-of-charge (http://sea-urchin.caltech.edu/software).

Scientists at the Sea Urchin Genome Resource also produce macroarrays, which are made available at cost of production to qualified researchers. These macroarrays are sets of six square nylon plates, 22 cm on a side, each holding 18,932 unique cDNA spots, laid out in duplicate by a robotic system. This setup allows researchers to interrogate more than 100,000 genes simultaneously by seeing where radioactively labeled test sequences bind to their complementary counterparts on the array. Another instrument then assesses each plate's two-dimensional image and records the exact location of any reactions. The identities of target clones can then be determined.

"Studying gene expression during embryogenesis calls for a global analysis," like that provided by the macroarrays, says Dr. Davidson. "We went to a lot of effort to get this technology, knowing how much we'd need it down the road."

Down the road lies the Sea Urchin Genome Project. As useful as the sea urchin is at present, Dr. Davidson says it will become even more valuable as the animals' genome is sequenced and comparisons can be made with the genomes of other organisms, including humans. Scientists already know that the spiny creatures are more closely related to humans than are those other staples of genetic research, the fruit fly and the nematode worm. Sea urchins are close enough to humans to make comparison worthwhile, yet distant enough to provide insight into the course of evolution over the last 540 million years.

In the end, the intensive work by Dr. Davidson's group describes an interlocking information network of regulatory genes and transcription factors operating during embryogenesis. "It's exceedingly complex and exceedingly important," says Dr. Davidson. "We have to understand gene regulatory networks, or we won't be able to truly understand development at all."

—Aaron Levin

For more information about the Sea Urchin Genome Resource, visit the Web site at http://sugp.caltech.edu, or contact resource co-investigator Dr. R. Andrew Cameron at 626-395-8421; fax: 626-793-3047; e-mail: acameron@caltech.edu.

The Sea Urchin Genome Resource is supported by the Division of Comparative Medicine of the National Center for Research Resources. To learn more about other NCRR-supported comparative medicine resources, see www.ncrr.nih.gov/comparative_med.asp.

Additional Reading

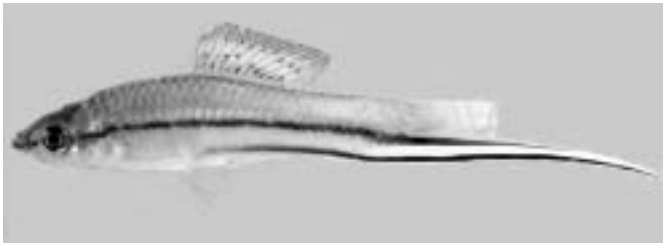
1. Davidson, E. H., Rast, J. P., Oliveri, P., et al., A genomic regulatory network for development. *Science* 295:1669-1678, 2002.
2. Cameron, R. A., Mahairas, G., Rast, J. P., et al., A sea urchin genome project: Sequence scan, virtual map, and additional resources. *Proceedings of the National Academy of Sciences USA* 97:9514-9518, 2000.

News from NCRR

Primate Centers Broaden Focus

Earlier this year, the nation's eight NCRR-supported Regional Primate Research Centers (RPRCs) were renamed National Primate Research Centers (NPRCs) to reflect their enhanced emphasis on providing nonhuman primates and related resources to biomedical scientists nationwide. NCRR broadened the scope of the centers because of increased demand for nonhuman primates in the study of AIDS, neurobiology, cardiology, and other research areas. The first seven primate research centers were established in the 1960s, and an eighth center was added to the network in 1999. Today this network, located around the country, maintains more than 24,000 nonhuman primates and provides nonhuman primate cells, tissues, organs, and biological fluids to investigators all over the United States and the world.

NCRR Funds Unique Fish Resource



NCRR has awarded a five-year, $1.7 million grant to the *Xiphophorus* Genetic Stock Center at Southwest Texas State University to help make the tropical fish *Xiphophorus* more widely available to biomedical researchers. As the only source of pedigreed *Xiphophorus* lines in the world, the Center maintains more than 60 genetic strains of the fish, which is an increasingly important animal model for biomedical studies.

The *Xiphophorus* Genetic Stock Center was founded more than six decades ago, following the discovery that crossing the platyfish *Xiphophorus maculatus* with the swordtail *Xiphophorus helleri* produced hybrids that developed cancers virtually identical to human skin cancer, or malignant melanoma. This led to a search for the genes responsible for the melanoma and the collection of wild *Xiphophorus* species from Central America. Meticulous breeding and analyses of the fish provided some of the earliest evidence that certain cancers can be inherited. In addition to its role in cancer research, *Xiphophorus* is also used for studying evolution of the immune system, neural systems that maintain balance, sex-determining genes, and brain distribution of chemicals that regulate growth and reproduction.

Originally housed in various locations in New York City, the *Xiphophorus* Genetic Stock Center was transferred in 1992 to Southwest Texas State University in San Marcos. Today the Center maintains representatives of all but 1 of the 23 known *Xiphophorus* species and houses nearly 6,500 fish. For more information about the *Xiphophorus* Genetic Stock Center, visit www.xiphophorus.org.

Genetically Modified Mouse Strains Available

The Mutant Mouse Regional Resource Centers (MMRRC) Program—established by NCRR in 1999 to maintain and distribute strains of scientifically valuable, genetically modified mice—now has 11 strains available for distribution. Nine of the strains are maintained as live colonies, while the other two strains are maintained in a cryopreserved archive. The strains can be used for studying cardiovascular disease, cancer, and bone matrix biology, among other subjects. The MMRRC Web site (www.mmrrc.org) provides details on available strains as well as a list of new strains under development. Also on the Web site is information about procedures for submitting new strains for preservation and distribution.

For mice that are maintained in a live colony, the MMRRC customarily supplies investigators with one or two breeder pairs. For mice that are maintained in the form of cryopreserved embryos or gametes, the MMRRC resuscitates the mice from the cryopreserved material before distribution. Fees are charged for distribution and cryo-recovery to defray costs for services, which include genetic quality control, phenotypic characterization of strains, and maintenance of a mouse resource database.

The MMRRC network currently includes four facilities located at the University of North Carolina at Chapel Hill; the University of California, Davis; Taconic Farms in New York, in collaboration with the State University of New York at Albany; and Harlan Sprague Dawley, Inc., in collaboration with the University of Missouri. The MMRRC Informatics Coordinating Center is at The Jackson Laboratory in Bar Harbor, Maine.

Crowley Receives Clinical Research Award

Dr. William F. Crowley, Jr., professor of medicine at Harvard Medical School and chief of the Reproductive Endocrine Unit at Massachusetts General Hospital, received the 14th Annual General Clinical Research Centers (GCRC) Award for Excellence in Clinical Research. The award recognizes outstanding clinical investigators who have conducted studies in a GCRC within the previous decade. Dr. Crowley was honored for his pioneering work in the neuroendocrine control of human reproduction. He accepted the award at the 2002 GCRC Program Directors' Meeting, held in Baltimore.

Dr. Crowley is known for developing long-acting gonadotropin-releasing hormone (GnRH) agonists as the standard treatment for precocious puberty. The agonists suppress release of the gonadotropin hormones that promote sexual development. Dr. Crowley also discovered that repeated administration of GnRH could induce sexual maturation in patients who had not undergone normal puberty because of inadequate gonadotropin secretion.

Primate Center Hosts Simian AIDS Symposium

About 300 researchers from around the world gathered in Monterey, California, for the 20th Annual Symposium on Nonhuman Primate Models for AIDS, held September 8-11. Symposium attendees discussed the latest advances enabled by studies of the simian immunodeficiency virus (SIV), which causes AIDS in monkeys and is closely related to the human immunodeficiency virus (HIV). Funded in part by NCRR, the symposium was hosted by the California National Primate Research Center (NPRC) at the University of California, Davis, where some of the earliest studies of simian AIDS and SIV began in the early 1980s. (For more information, see the *NCRR Reporter*, Spring 2002, pp. 19-21.)

Among the symposium presentations were several on potential vaccines designed to protect nonhuman primates from infection with SIV or combination SIV-HIV (SHIV) strains. One approach, developed by scientists at the New England NPRC, involves immunizing animals with an SIV strain that cannot replicate, which minimizes the chances that the vaccine might cause the disease it is supposed to prevent. Another approach, developed by scientists at the Washington NPRC and their colleagues, involves vaccinating monkeys with immune cells expressing SHIV antigens in order to generate immune responses against these antigens. Presentations from the symposium will be published in a forthcoming issue of the *Journal of Medical Primatology*.

NCRR Establishes a Chimpanzee Sanctuary

NCRR has awarded a contract to Chimp Haven, Inc.—a private, nonprofit organization—to establish and operate a sanctuary for chimpanzees no longer needed for biomedical research. Such a sanctuary was mandated by The Chimpanzee Health Improvement, Maintenance, and Protection (CHIMP) Act, passed in December 2000, to provide lifetime care for federally owned or supported chimpanzees retired from research. Over the term of the 10-year contract, NCRR will provide approximately $19 million in total costs, and Chimp Haven will contribute approximately $4 million toward direct costs. In addition, NCRR has awarded a $5 million grant to Chimp Haven to support construction of the new sanctuary near Shreveport, Louisiana.

In Spring 2004, the sanctuary is expected to initially provide housing in a free-ranging environment for approximately 75 chimpanzees. If more chimpanzees are transferred from research facilities to the sanctuary, Chimp Haven is authorized to either expand its sanctuary facility or serve as a primary contractor to additional qualified sanctuary sites, which will be maintained by subcontractors.

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NCRR Releases New GCRC and Clinical Career Fact Sheets

NCRR has issued two new fact sheets that describe the network of General Clinical Research Centers (GCRCs) and career development programs designed to attract professionals to clinical research. The 4-page *General Clinical Research Centers Fact Sheet,* written especially for potential GCRC users, provides an overview of the GCRC Program, which was authorized by Congress in 1959 to provide clinical investigators with the infrastructure necessary to conduct sophisticated patient-oriented research. GCRCs began as discrete inpatient units in university hospitals but now also include outpatient facilities. The fact sheet outlines GCRC funding and management, investigator access, and the roles and responsibilities of GCRC team members.

The 4-page *Clinical Research Career Development Fact Sheet* describes the range of NCRR-supported career development programs based at the GCRCs. These grant programs are intended to attract talented medical students, physicians, dentists, and similar professionals to clinical research or to help clinical investigators transition to independent research careers. The fact sheet also briefly describes the NIH Loan Repayment Program.

These and other NCRR fact sheets are available on NCRR's Web site at www.ncrr.nih.gov/publications.asp and can be obtained free-of-charge from the Office of Science Policy and Public Liaison, NCRR/NIH, 6705 Rockledge Drive, Bethesda, MD 20892-7965; phone: 301-435-0888; fax: 301-480-3558; e-mail: info@ncrr.nih.gov.

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